JACC: CARDIOVASCULAR INTERVENTIONS VOL. 1, NO. 3, 2008

ISSN 1936-8798/08/$34.00
PUBLISHED BY ELSEVIER INC. DOI: 10.1016/j.jcin.2008.01.009

Abnormal Vasomotor Function of Porcine Coronary Arteries Distal to Sirolimus-Eluting Stents

Jinsheng Li, MD, PHD, Refat Jabara, MD, Lakshmana Pendyala, MD, Yoritaka Otsuka, MD, Toshiro Shinke, MD, Dongming Hou, MD, PHD, FACC, Keith Robinson, PHD, FACC, Nicolas Chronos, MD, FACC

Atlanta, Georgia

Objectives This study sought to determine vasomotor functional responses of conduit coronary artery distal to bare-metal stents (BMS), polymer-only stents (POLY), and sirolimus-eluting stents (SES) in a clinically relevant animal model.

Background Drug-eluting stents (DES) reduce in-stent restenosis, and also affect neointima formation and vascular remodeling in downstream coronary segments. Whether distal artery vasomotor function is also influenced by DES has not been determined.

Methods Pigs ($n = 12$) received coronary stent implants, and hearts were harvested at 1 month. Arterial segments ≥15 mm distal to stents were excised and studied in an organ-chamber apparatus. Endothelium-dependent and endothelium-independent relaxation and contraction to classical agonists were measured.

Results The SES showed increased lumen area and reduced neointima; abnormal vasomotor function of conduit arteries distal to SES also was observed. Contraction to endothelin-1 was significantly enhanced for SES compared with both BMS and POLY. Endothelium-dependent relaxation to a maximal dose of substance P was attenuated for SES compared with both BMS and POLY (46 ± 6% vs. 71 ± 3% and 78 ± 3%, respectively, $p < 0.001$). Endothelium-independent relaxation to sodium nitroprusside was potentiated for SES, compared with BMS and POLY (100 ± 5% vs. 69 ± 7% and 77 ± 5%, respectively, $p = 0.02$).

Conclusions Stent-based local delivery of sirolimus profoundly inhibited neointima formation but caused vasomotor dysfunction in distal conduit vessel segments. These observations suggest that distal coronary vasospasm may be more readily evoked in the presence of DES and contribute to pathophysiological sequela. (J Am Coll Cardiol Intv 2008;1:279–85) © 2008 by the American College of Cardiology Foundation

From the Saint Joseph's Translational Research Institute/Saint Joseph's Hospital of Atlanta, Georgia.
Manuscript received November 16, 2007; revised manuscript received January 23, 2008, accepted January 29, 2008.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 1, NO. 3, 2008
JUNE 2008:279–85

Development and widespread application of drug-eluting stents (DES) has provided a novel and efficacious treatment for coronary artery disease, allowing programmable localized elution of drugs to inhibit neointima formation and thereby reduce in-stent restenosis compared with bare-metal stents (BMS) (1–7). However, concerns have arisen regarding adverse effects of DES in some patients, including delayed healing, hypersensitivity reactions, and impact of eluted drug on downstream tissue. Delayed or incomplete re-endothelialization is an important predictor of late stent thrombosis (LST), which is increasingly apparent for DES in real-world applications. Prolonged dual antiplatelet therapy has been recommended to reduce LST, a rare but potentially life-threatening complication of DES that has become an increasingly controversial issue for interventional cardiology (8–10). As a result, the widespread use and long-term safety of DES have been questioned.

Abbreviations and Acronyms

BMS = bare-metal stent(s)
DES = drug-eluting stent(s)
ET = endothelin
eNOS = endothelial nitric oxide synthase
IEL = internal elastic lamina
L-NAME = N^{W}-nitro-$_{L}$-arginine methyl ester
LST = late stent thrombosis
$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$
POLY = polymer-only coated stent(s)
SES = sirolimus-eluting stent(s)
SNP = sodium nitroprusside

Several reports have recently shown that sirolimus-eluting stents (SES) are associated with impaired endothelial vasodilator function in peristent arterial segments as late as 6 months after implant (11). On the other hand, subjects who received BMS showed normal endothelial function in peristent segments. Paradoxical vasoconstriction was induced in response to exercise (12) and pharmacological stress (13,14), including severe vasoconstriction in distal coronary segments. From major clinical trials, DES have been found to inhibit neointima formation at the distal stent edge and in the coronary segment downstream to the stent, but have less impact in the proximal site (15,16); together these observations point to a cumulative downstream watershed impact of upstream-eluted drug compound.

Nitric oxide (NO) released primarily from the vascular endothelium plays pivotal roles in coronary vasomotor function, inflammation, thrombosis, atherosclerosis, and arterial neointima formation (17–19). Dysregulation of NO synthesis or bioavailability is associated with paradoxical vasoconstriction, procoagulant state, and increased inflammation, and may play a role in LST. Comprehensive in vitro vasomotor function analysis in conduit artery segments distal to SES implants has not been previously reported. The purpose of the present study was to assess vasomotor function of coronary segments distal to SES in a clinically relevant porcine coronary artery model (20,21).

Methods

All experiments and animal care conformed to federal guidelines and were approved by the Institutional Animal Care and Use Committee. Twelve castrated male and female Yorkshire hybrid domestic pigs (Sus scrofa) were used, and received daily oral antiplatelet medication, with clopidogrel 300 mg and aspirin 81 mg for 3 days before stent implantation then clopidogrel 75 mg and aspirin 81 mg daily until termination. Body weight was 31 ± 2 kg and 39 ± 2 kg at the times of the stent implant and at 1 month follow-up, respectively.

Stent implant procedure. Stents were implanted according to standard procedures. Vessel diameter at the target site was measured by quantitative coronary angiography. Bare-metal stents (BMS) (ACS multilink 3.0/28 mm and Penta multilink 3.5/28 mm, Guidant, Santa Clara, California), polymer-only coated stents (POLY) (ChronoFlex AL, 3.0/28 mm. ChronoFlex AL is an aliphatic, ether-free, polycarbonate thermoplastic polymer that belongs to a family of biodurable thermoplastic polyurethane elastomers, CardioTech, Wilmington, Massachusetts), and SES (Cypher, 3.0/23 and 3.5/28 mm, Cordis, Miami, Florida) were implanted in 3 coronary arteries per pig by randomized assignment to anatomic location. The stent was advanced to the selected location and deployed by inflation of the balloon catheter to a pressure appropriate to oversize by approximately 15% relative to baseline vessel diameter. Animals were recovered and returned to routine care.

Histology. Three pigs were terminated 1 month post-stenting; hearts were harvested and perfused with saline and then perfusion-fixed with a 5% formalin/1.25% glutaraldehyde mixture. The next day, the stented left anterior descending artery, left circumflex artery, and right coronary artery were excised. The stented vessels (3 BMS, 3 POLY, and 3 SES) were dehydrated in graded ethanol series to 100% then embedded in methyl methacrylate. Sections were cut from the proximal, middle, and distal stent regions using a heavy-duty microtome and collected on glass slides. Adjacent or near-adjacent sections were stained with hematoxylin-eosin and Verheoff-Masson elastin-trichrome. Histological sections were prepared and evaluated for the extent of stent-induced coronary vessel injury and response.

Each section was imaged by charge-coupled device camera at an appropriate low magnification. Histomorphometric analysis was performed by computerized planimetry on proximal, mid, and distal sections. The following measurements were made: neointimal thickness (lumen to each stent strut), luminal area, internal elastic lamina (IEL) area, and external elastic lamina area. The areas of the neointima and media were obtained by subtraction of the lumen area from the IEL and IEL from external elastic lamina, respectively. The histological percent area stenosis ([1 − (luminal area/IEL area)] × 100) was calculated.

Vascular function study. Also at 1 month after stent implant, the remaining 9 pigs were terminated; hearts were harvested and placed in ice-cold Krebs solution. Coronary artery segments beyond 15 mm distal to stent implants were cleaned of loose fat and connective tissue and cut into 4-mm long rings (Fig. 1). One ring from each segment (6 BMS, 5 POLY, and 6 SES) was suspended in an individual organ chamber (Radnoti Glass Technology, Monrovia, California) filled with 17 ml freshly prepared Krebs solution with the following composition (mM): NaCl 120, $MgSO_4$ 1.17, KH_2PO_4 1.18, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, glucose 5.5, and 10 μM indomethacin at pH 7.4. The solution was continuously bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C. Vascular rings were gradually stretched to a basal tension of 3 g, which was continuously adjusted over approximately 90 min until vessel tension stabilized. Vessels were subjected to the same passive tension of 3 g throughout the remainder of the study. Krebs buffer was changed every 15 min during the equilibration period.

Vessel responsiveness was tested with 40 and 100 mM KCl. Rings were pre-constricted with a single dose (5 μM) of prostaglandin F2α ($PGF_{2\alpha}$) until they reached a stable plateau (approximately 7 min). Arterial rings were then exposed to a series of endothelium-dependent and endothelium-independent vasodilators. First, concentration response to substance P (0.01 to 100 pM) and sodium nitroprusside (SNP; 0.001 to 10 μM) was determined. After incubation with 100 μM N^W-nitro-$_L$-arginine methyl ester (L-NAME) (a competitive inhibitor of NO synthase) for 45 min, substance P concentration response was repeated. Rings were then contracted with 0.1 μM endothelin-1 (ET-1) at the end of each experiment. Vessels were washed for 45 min between each serial concentration-response determination. Isometric tension was digitized, acquired, and analyzed using a Ponemah Tissue Platform System (Gould Instrument System, Valley View, Ohio).

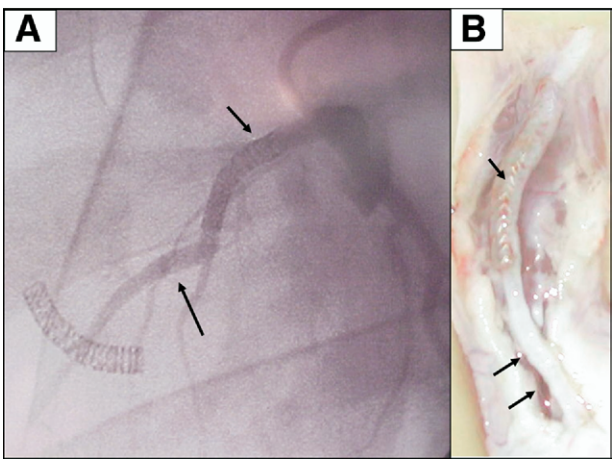

Figure 1. Angiography and Macroscopy of Coronary Segments

(A) Coronary angiography 1 month after stent implant in LAD **(top arrow)**; segments studied were the distal conduit vessel **(bottom arrow)**. **(B)** Macroscopy at tissue harvest showing the LAD stent **(top arrow)** and relation to distal conduit vessel segment **(bottom arrows)** harvested and analyzed in the organ chamber apparatus. LAD = left anterior descending artery.

Statistical analysis. Data are presented as mean ± standard error, and were assessed using one-way analysis of variance to test for the effect of upstream stent type on each dependent variable. If a significant treatment effect was observed, multiple comparisons using Tukey test were made to identify between-groups differences. No corrections were made for multiple analysis of variance on the same variables at different concentrations. For certain variables, Student *t* tests were used to compare group means. Significance was established at the 95% confidence level ($p < 0.05$).

Results

Macroscopic findings and histomorphometry in stented segments. Baseline lumen diameter of target coronary segments was similar among BMS, POLY, and SES groups (2.84 ± 0.12 mm, 2.81 ± 0.08 mm, and 2.75 ± 0.09 mm, respectively, $p = 0.806$). Angiography showed appropriate stent deployment without complications in all vessels. No distal edge dissection or vasospasm was observed during any stent implant procedure, for any stent type, that might influence subsequent coronary vasomotor function.

At tissue harvest 1 month post-implant, there were no grossly visible myocardial infarctions. Macroscopic examination revealed loose fat and connective tissue surrounding the stented BMS and POLY vessels. Pericoronary tissue around SES was denser and more difficult to dissect than BMS and POLY stents. These morphologies were not present at sites proximal or distal to the stent segment in any of the stent groups.

Representative histological sections of stented segments of BMS, POLY, and SES are shown in Figure 2. Neointimal thickness was 0.17 ± 0.04 mm for SES group compared with 0.31 ± 0.06 mm for BMS and 0.62 ± 0.13 mm for POLY, respectively, $p = 0.008$.

Vasomotor function distal to stent implants. Contractile responses of downstream coronary segments to 40 and 100 mM KCl did not differ according to stent type. Baseline ratio of 5 μM $PGF_{2\alpha}$-induced contraction to 40-mM KCl-induced contraction was also similar among groups (0.31 ± 0.05% vs. 0.32 ± 0.07% and 0.40 ± 0.12%, $p = 0.75$). However, in the presence of the NO synthetase inhibitor L-NAME, contraction to $PGF_{2\alpha}$/40-mM KCl was potentiated for SES (0.93 ± 0.18% vs. 0.31 ± 0.05%, $p = 0.008$) (Fig. 3A). Contraction to ET-1 compared with contraction to 40-mM KCl was greater for SES than for BMS and POLY (1.67 ± 0.15% vs. 1.15 ± 0.03% and 1.17 ± 0.09%, respectively, $p = 0.021$) (Fig. 3B).

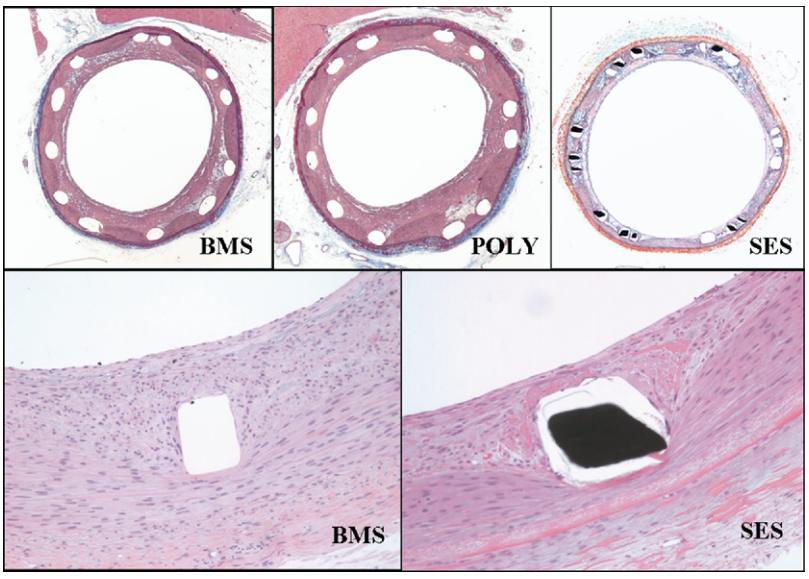

Figure 2. Histologic Sections of Stented Arteries

Illustrative histological sections from BMS, POLY, and SES groups. BMS = bare-metal stent(s); POLY = polymer-only coated stent(s); SES = sirolimus-eluting stent(s).

BMS and POLY arteries relaxed in a dose-dependent manner to substance P, an endogenous endothelium-dependent receptor-mediated vasodilator (Fig. 4A). However, for vessels distal to SES implants the cumulative concentration curve was significantly shifted to the right. The SES vessels showed significantly reduced relaxation at maximal substance P concentration (46 ± 6% vs. 71 ± 3% and 78 ± 3% for SES, BMS, and POLY respectively, $p < 0.001$).

Sodium nitroprusside, an endothelium-independent vasodilator, induced concentration-dependent relaxation of coronaries distal to all 3 stent types (Fig. 4B). Unlike substance P, SNP-induced maximum relaxation was higher for SES than BMS and POLY (100 ± 5% vs. 73 ± 7% and 77 ± 5%, respectively, $p < 0.02$).

After NO blockade with L-NAME, concentration-dependent relaxation to substance P was significantly blunted for BMS and POLY to a similar extent (Figs. 5A and 5B). For vessel segments distal to SES, relaxation to substance P in the presence of L-NAME was augmented compared with BMS and POLY groups (Fig. 5C).

Discussion

In this study, we investigated endothelial function in coronary artery segments distal to BMS, POLY, and SES sites at 1 month after implantation. Neointimal formation was markedly suppressed in SES segments, consistent with previous reports (22-25). Distal conduit arteries devoid of any direct mechanical injury showed vasomotor dysfunction distal to SES, but not BMS or POLY stents. Increased contractile and endothelium-independent relaxation responses as well as reduced endothelium-dependent relaxation were noted after a period known to comprise the duration of nearly complete drug release.

The SES elute more than 80% of constituent drug within 30 days. In one study after implantation, about 90% of sirolimus was released into the vessel wall and immediately adjacent tissue, and 7% into remote areas of the heart (26). This indicates that the amount of sirolimus directly eluted to distal arterial segments and myocardium via the conduit artery luminal circulation was likely to be relatively small, and suggests that most of the drug taken up into cardiac tissue was transferred by direct contact with the arterial wall, which may have served as a reservoir for subsequent transfer via diffusion, or the vasa vasorum. By such a route, the eluted compound could have pharmacological and side effects several centimeters from the stent site (27).

Several groups have shown that SES are associated with vasomotor dysfunction in the peristent area (approximately 5 mm) in vivo at late time points. However, these studies were carried out using human patients, with vasomotor evaluation using angiography. Several factors affecting endothelial function in vivo could confound these analyses, including existing arteriosclerotic disease, catheter-based intervention, medicinal therapies with direct or indirect effects on endothelial function, sirolimus-releasing period, healing response, and local hemodynamics. Togni et al. (12) pointed out that testing endothelial function in human

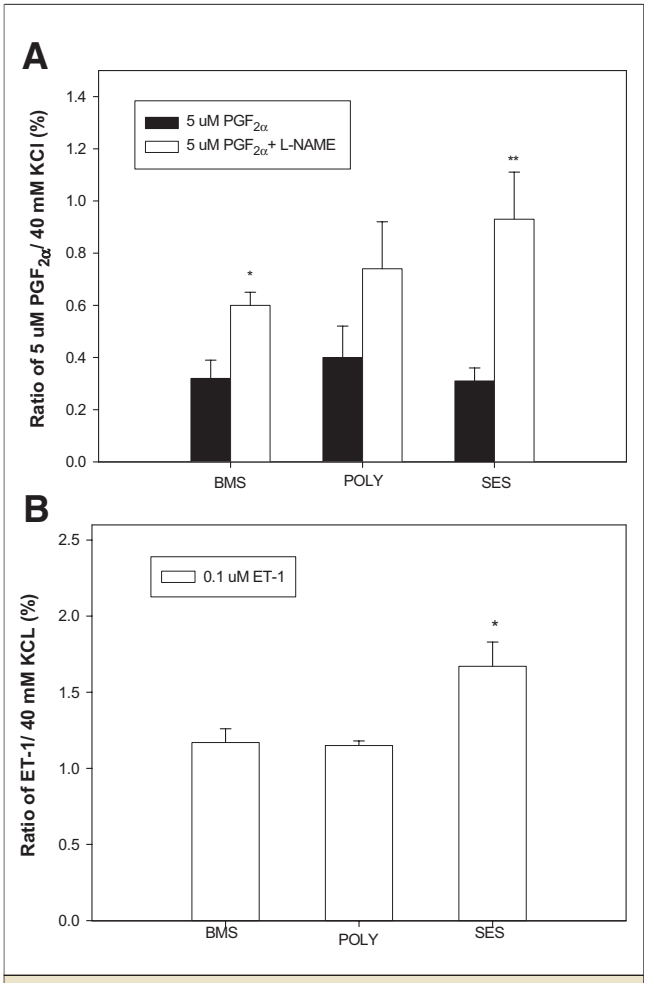

Figure 3. Contractile Responses of Coronary Segments Distal to Stents

Contractile responses to constriction agonists for coronary arteries distal to BMS, POLY, and SES stents. **(A)** In the presence of N^{W}-nitro-$_{L}$-arginine methyl ester (L-NAME), contractile response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was increased for SES (*p = 0.008). **(B)** The contractile response to endothelin (ET)-1 was also increased for SES (*p = 0.021). Abbreviations as in Figure 2.

patients is technically difficult, and few comparative data are available. There are no comprehensive animal studies addressing SES-associated endothelial dysfunction published before the present report.

In our study, we found that endothelium-dependent relaxation to substance P was significantly decreased at higher concentrations distal to SES. Endothelium-independent relaxation of the distal segments was maintained in all 3 stent types, and in fact paradoxically increased at a maximal dose of SNP in SES-stented arteries. Removal of endothelium has previously been found to enhance vasomotor sensitivity to NO donors, and thereby increased SNP-induced vasorelaxation (28). The NO generation by vascular endothelium attenuates the NO-donor (SNP) response, and a competitive interaction between endothelial NO and SNP was thought responsible for this effect (29,30).

Several groups observed exercise-induced and drug-induced vasoconstriction with normal nitroglycerine-induced vasodilatation in segments distal to SES 6 months after implant in humans (11–14); our findings corroborate the first result but are at variance with the latter. This difference could originate from a variety of mechanisms including species differences, analytical methods (in vivo angiographic vs in vitro organ chamber), and location (distal stent segment vs approximately 15 mm distal to stent site). Our results documenting decreased endothelium-dependent relaxation and increased endothelium-independent relaxation distal to Cypher implants support the notion that

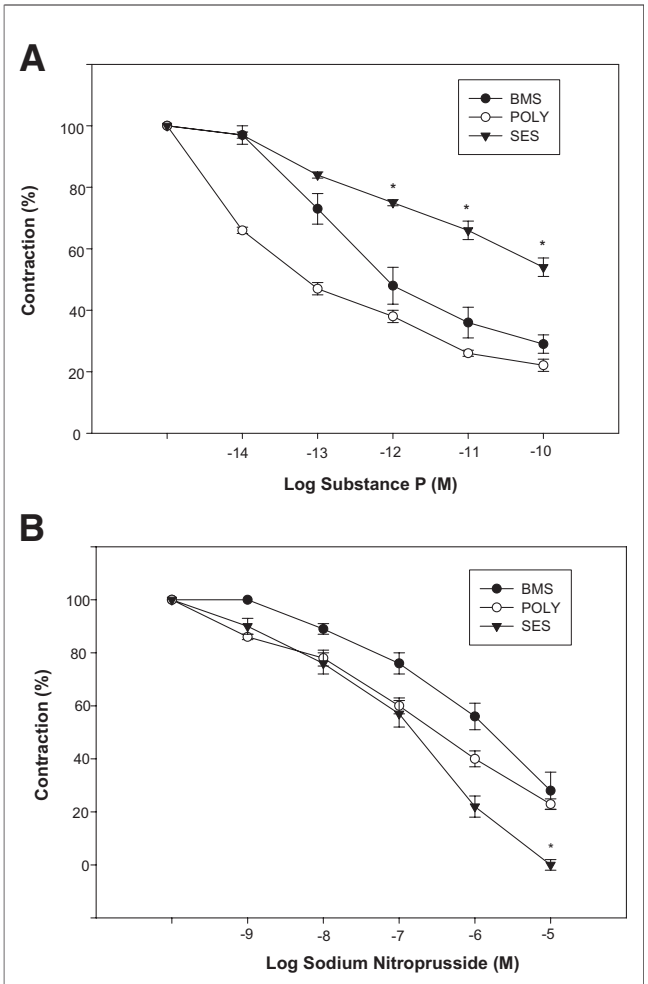

Figure 4. Relaxation Responses of Coronary Segments Distal to Stents

Cumulative concentration–relaxation curves for coronary artery segments distal to BMS, POLY, and SES. **(A)** Relaxation to higher concentrations of endothelium–dependent vasodilator substance P was inhibited in coronary segments distal to SES compared with BMS and POLY (*p < 0.05). **(B)** Relaxation to the highest concentration of the endothelium-independent dilator sodium nitroprusside was higher for SES compared with BMS and POLY (*p < 0.02). Abbreviations as in Figures 1 and 2.

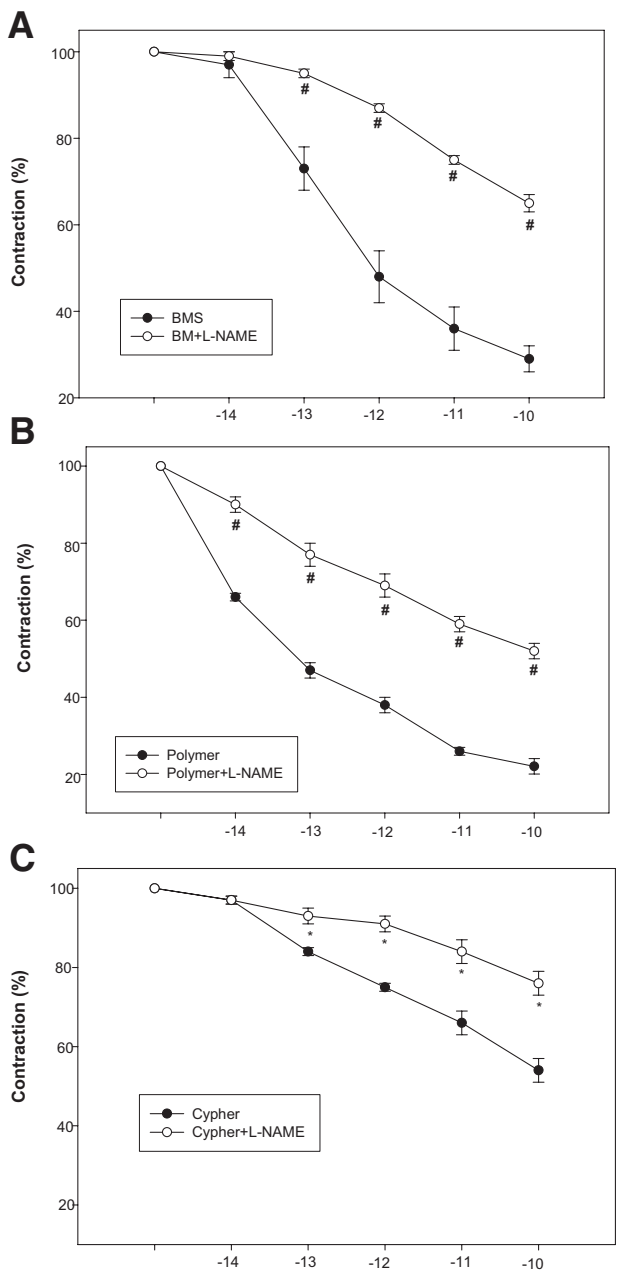

Figure 5. Effect of Nitric Oxide Synthase Blockade on Endothelium-Dependent Relaxation Response of Coronary Segments Distal to Stents

In the presence of nitric oxide synthetase inhibitor L-NAME, relaxations to endothelium-dependent receptor-mediated vasodilator agonist substance P were inhibited to a greater degree in BMS (A) than in POLY (B) than in SES (C) ($*p < 0.05$, $^{\#}p < 0.01$). Abbreviations as in Figures 2 and 3.

there is decreased NO bioavailability from the endothelial layer in the segments of conduit coronary artery distal to the sirolimus-eluting implants. Thus, it appears that SES can exert very potent effects on adjacent arterial segments, particularly distal segments, where it would be anticipated that drug released from the stents on the luminal aspect might enter the circulation at especially high concentrations in the flow boundary layer, where the tissue interface consists of the vascular endothelium.

Contractile responses to KCl and $PGF_{2\alpha}$ were similar among the stent types. Contractions to ET-1 were significantly enhanced for SES compared with BMS and POLY stents. In the presence of the endothelial nitric oxide synthase (eNOS) inhibitor L-NAME, contraction to PGF was increased. Furthermore, in the presence of L-NAME, the minimal substance P-induced relaxations in Cypher arteries were more profoundly blocked than in the absence of the NOS inhibitor. These data suggest that both endogenous vasoconstrictor substances as well as eNOS were enhanced in conduit vessel segments distal to the Cypher stent implant. Thus, an augmented eNOS activity may be present as a compensatory mechanism to alleviate the dysfunctional vasomotion.

Sirolimus itself been reported to inhibit endothelial function in vitro. Mohacsi et al. (31) reported that sirolimus-inhibited growth factor induced proliferation of the cultured endothelial and smooth muscle cells. Jeanmart et al. (32) also showed endothelial dysfunction in porcine coronary arteries with an in vitro model of drug incubation in Krebs-bicarbonate solution. They reported that porcine epicardial arteries exposed to sirolimus had severe impairment of relaxant responses to serotonin and bradykinin, and suggested that sirolimus had a direct adverse effect on endothelial function. Impairment of endothelial recovery may also have adverse long-term impacts such as LST, lesion recurrence, and constrictive vascular remodeling (33,34).

Our results show that although effective to inhibit neointima formation, drugs eluted from stents can adversely affect vasomotor function of downstream coronary segments. The pattern of changes we observed for Cypher stents suggests that at any given level of an endogenous humoral vasoactive agent, coronary arteries distal to the DES site will constrict more readily than for sites distal to BMS. This lower set point for contraction would be anticipated to have adverse effects on distal myocardial perfusion and regional myocardial function as well as to limit blood flow velocity and exacerbate nonlaminar flow within the stented segment, potentially increasing thrombotic and inflammatory tendencies. Longer-term studies are needed to verify and elucidate the effect of the drugs on the vascular bed.

Study limitations. The small numbers of animal subjects should be considered and the statistical analysis of data viewed accordingly when interpreting these results. We acknowledge the potential limitations imparted by the utilization of different stent platforms for each group. The differences in stent material and design may introduce additional and potentially confounding variables. In addition, normal porcine coronary arteries may not necessarily predict similar effects as in atherosclerotic human vessels.

However, similar studies in normal porcine coronary arteries with DES have been used extensively and successfully as the basis for DES clinical research and development.

Conclusions

The SES may cause endothelial and vasomotor dysfunction of the coronary artery segments distal to implant sites in pig coronary arteries. This may contribute to increased late thrombotic adverse events through diverse pathophysiological mechanisms. The long-term clinical consequences of this phenomenon remain to be established.

Acknowledgments
The authors thank Cindy Baranowski for her expert technical assistance with the histologic section preparation; Bobby Price, Melissa Moon, David Robinson, and Ghulam Abbas for their support with the stent implant procedures; and Addrena Taylor for her assistance with the necropsy and tissue preparation.

Reprint requests and correspondence: Dr. Refat Jabara, Saint Joseph's Translational Research Institute/Saint Joseph's Hospital of Atlanta, 5673 Peachtree Dunwoody Road, Suite 675, Atlanta, Georgia 30342. E-mail: rjabara@sjha.org.

REFERENCES

1. Spaulding C, Daemen J, Boersma E, Cutlip DE, Serruys PW. A pooled analysis of data comparing sirolimus-eluting stents with bare-metal stents. N Engl J Med 2007;356:989–97.
2. Stone GW, Moses JW, Ellis SG, et al. Safety and efficacy of sirolimus- and paclitaxel-eluting coronary stents. N Engl J Med 2007;356:998–1008.
3. Lagerqvist B, James SK, Stenestrand U, Lindback J, Nilsson T, Wallentin L. Long-term outcomes with drug-eluting stents versus bare-metal stents in Sweden. N Engl J Med 2007;356:1009–19.
4. Mauri L, Hsieh W, Massaro JM, Ho KKL, D'Agostino R, Cutlip DE. Stent thrombosis in randomized clinical trials of drug-eluting stents. N Engl J Med 2007;356:1020–9.
5. Kastrati A, Mehilli J, Pache J, et al. Analysis of 14 trials comparing sirolimus-eluting stents with bare-metal stents. N Engl J Med 2007;356:1030–9.
6. Maisel WH. Unanswered questions—drug-eluting stents and the risk of late thrombosis. N Engl J Med 2007;356:981–4.
7. Farb A, Boam AB. Stent thrombosis redux—the FDA perspective. N Engl J Med 2007;356:984–7.
8. Iakovou I, Schmidt T, Bonizzoni E, et al. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. JAMA 2005;293:2126–30.
9. Moreno R, Fernández C, Hernández R, et al. Drug-eluting stent thrombosis: results from a pooled analysis including 10 randomized studies. J Am Coll Cardiol 2005;45:954–9.
10. Ong AT, Hoye A, Aoki J, et al. Thirty-day incidence and six-month clinical outcome of thrombotic stent occlusion after bare-metal, sirolimus, or paclitaxel stent implantation. J Am Coll Cardiol 2005;45:947–53.
11. Brott BC, Anayiotos AS, Charpman GD, Anderson PG, Hillegass WB. Severe, diffuse coronary artery spasm after drug-eluting stent placement. J Invasive Cardiol 2006;18:584–92.
12. Togni M, Windecker S, Cocchia R, et al. Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction. J Am Coll Cardiol 2005;46:231–6.
13. Hofma SH, van der Giessen WJ, van Dalen BM, et al. Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. Eur Heart J 2006;27:166–70.
14. Fuke S, Maekawa K, Kawamoto K, et al. Impaired endothelial vasomotor function after sirolimus-eluting stent implantation. Circ J 2007;71:220–5.
15. Kipshidze N, Leon MB, Tsapenko M, Falotico R, Kopia GA, Moses J. Update on sirolimus drug-eluting stents. Curr Pharm Design 2004;10:337–48.
16. Weissman NJ, Ellis SG, Grube E, et al. Effect of the polymer-based, paclitaxel-eluting TAXUS Express stent on vascular tissue responses: a volumetric intravascular ultrasound integrated analysis from the TAXUS IV, V, and VI trials. Eur Heart J 2007;13:1574–82.
17. Marx SO, Jayaraman T, Go LO, Marks AR. Rapamycin-FKBP inhibits the cell cycle regulators of proliferation in the vascular smooth muscle cells. Circ Res 1995;76:412–7.
18. Fukuda D, Sata M, Tanaka K, Nagri R. Potent inhibitory effect of the sirolimus on the circulating vascular progenitor cells. Circulation 2005:111:926–31.
19. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000;87:840–4.
20. Schwartz RS, Edelman ER, Carter A, et al. Drug-eluting stents in preclinical studies: recommended evaluation from a consensus group. Circulation 2002;106:1867–73.
21. Schwartz RS, Chronos NAF, Virmani R. Preclinical restenosis models and drug-eluting stents. Still important, still much to learn. J Am Coll Cardiol 2004;41:1373–85.
22. Klugherz BD, Llanos G, Lieuallen W, et al. Twenty-eight-day efficacy and pharmacokinetics of the sirolimus-eluting stent. Coron Artery Dis 2002;13:183–8.
23. Carter AJ, Aggarwal M, Kopia GA, et al. Long-term effects of polymer-based, slow-release, sirolimus-eluting stents in a porcine coronary model. Cardiovasc Res 2004;63:617–24.
24. Lower HC, Oesterle SN, Khachigian LM. Coronary in-stent restenosis: current status and future strategies. J Am Coll Cardiol 2002;39:183–93.
25. Suzuki T, Kopia G, Hayashi S, et al. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. Circulation 2001;104:1188–93.
26. Yu MY, Gao RL, Jiang J, et al. Pharmacokinetics of rapamycin-eluting stents in miniswine coronary model. Chin Med J 2004;117:1459–63.
27. Gössl M, Rosol M, Malyar NM, et al. Functional anatomy and hemodynamic characteristics of vasa vasorum in the walls of porcine coronary arteries. Anat Rec Part A 2003;272:526–37.
28. Thorin E. Influence of nitric oxide synthase inhibition and endothelin-1 receptor blockade on acetylcholine-induced coronary artery contraction in vitro in dilated and ischemic cardiomyopathies. J Cardiovasc Pharmacol 2001;38:90–8.
29. Moncada S, Rees DD, Schulz R, Palmer RM. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. Proc Natl Acad Sci USA 1991;88:2166–70.
30. Dinerman JL, Lawson DL, Mehta JL. Interactions between nitroglycerin and endothelium in vascular smooth muscle relaxation. Am J Physiol 1991;260:H698–H701.
31. Mohacsi PJ, Tuller D, Hulliger B, Winjgaard PLJ. Different inhibitory effects of the immunosuppressive drugs on the human and rat aortic smooth muscle and endothelial cell proliferation stimulated by platelet derived growth factor. J Heart Lung Transplant 1997;16:484–92.
32. Jeanmart H, Malo O, Carrier M, Nickner C, Desjardines N. Comparative study of cyclosporine vs newer immunosuppresants mycophenalate mofetil and rapamycin on the coronary endothelial function. J Heart Lung Transplant 2002;21:990–8.
33. Schwartz RS, Chronos NA, Virmani R. Preclinical restenosis models and drug eluting stents: still important, still much to learn. J Am Coll Cardiol 2004;44:1373–85.
34. McFadden EP, Stabile E, Regar E, et al. Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy. Lancet 2004;364:1519–21.